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Indian Standard

ANIMAL FEEDING STUFFS — DETERMINATION OF CRUDE FIBRE CONTENT — METHOD WITH INTERMEDIATE FILTRATION

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

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Price Group 5

Livestock Feeds, Equipment and Systems Sectional Committee, FAD 5

NATIONAL FOREWORD

This Indian Standard which is identical with ISO 6865 : 2000 'Animal feeding stuffs — Determination of crude fibre content — Method with intermediate filtration' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Livestock Feeds, Equipment and Systems Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker in the International Standards, while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards, which are to be substituted in their respective places, are listed below along with their degree of equivalence for the editions indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 3696 : 1987 Water for analytical laboratory use — Specification and test methods	IS 1070 : 1992 Reagent grade water (*third revision*)	Technically Equivalent
ISO 6498 : 1998 Animal feeding stuffs — Preparation of test samples	IS 14831 : 2000 Animal feeding stuffs — Preparation of test samples	Identical

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

ANIMAL FEEDING STUFFS — DETERMINATION OF CRUDE FIBRE CONTENT — METHOD WITH INTERMEDIATE FILTRATION

1 Scope

This International Standard specifies a method with intermediate filtration for the determination of the crude fibre content. A manual procedure and a semi-automatic procedure are described.

The method is applicable to animal feeding stuffs with a crude fibre content greater than 10 g/kg.

NOTE For animal feeding stuffs with a crude fibre content equal to or less than 10 g/kg, the method described in ISO 6541 [7] may be used.

This International Standard is also applicable to cereals and pulses.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative documents referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 6498:1998, *Animal feeding stuffs — Preparation of test samples.*

3 Term and definition

For the purposes of this International Standard, the following term and definition apply.

3.1
crude fibre content
loss in mass resulting from ashing of the dried residue obtained after acid and alkaline digestion of the sample by the procedure described in this International Standard, divided by the mass of the test portion

NOTE The crude fibre content is expressed in grams per kilogram. It may also be expressed as a mass fraction in percent.

4 Principle

The test portion is treated with boiling dilute sulfuric acid. The residue is separated by filtration, washed and then treated with boiling potassium hydroxide solution. The residue is separated by filtration, washed, dried, weighed and then ashed. The loss in mass resulting from ashing corresponds to the mass of crude fibre in the test portion.

5 Reagents and materials

Use only reagents of recognized analytical grade.

5.1 **Water,** complying with at least grade 3 in accordance with ISO 3696.

5.2 **Hydrochloric acid,** $c(\mathrm{HCl}) = 0{,}5$ mol/l.

5.3 **Sulfuric acid,** $c(\mathrm{H_2SO_4}) = (0{,}13 \pm 0{,}005)$ mol/l.

5.4 **Potassium hydroxide solution,** $c(\mathrm{KOH}) = (0{,}23 \pm 0{,}005)$ mol/l.

5.5 **Acetone.**

5.6 **Filter aid:** sea sand , or Celite®[1] 545, or material of an equivalent quality.

Before use, treat sea sand with boiling hydrochloric acid [$c(\mathrm{HCl}) = 4$ mol/l], wash with water until free from acid and heat at a temperature of (500 ± 25) °C for at least 1 h.

Before use, heat other filter aids at a temperature of (500 ± 25) °C for at least 4 h.

5.7 **Antifoam agent,** for instance *n*-octanol.

5.8 **Light petroleum,** boiling range 40 °C to 60 °C.

6 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.1 **Grinding device,** capable of grinding the sample so that it passes completely through a **sieve** with 1 mm apertures.

6.2 **Analytical balance,** with accuracy of at least 0,1 mg.

6.3 **Filter crucibles,** of quartz, porcelain or hard glass, with fused sintered filter plate with a pore size of 40 µm to 100 µm (porosity grade P 100 according to ISO 4793:1980 [1]).,

Before using for the first time, carefully and gradually heat a new filter crucible to a temperature not exceeding 525 °C and leave for a few minutes at (500 ± 25) °C.

Stainless-steel crucibles with stainless-steel sieve plates with an aperture size of 90 µm of identical performance characteristics may be used as an alternative.

6.4 **Porcelain sieve plates.**

6.5 **Incineration dishes.**

6.6 **Beakers** or **conical flasks,** of 500 ml capacity, provided with a suitable cooling device, for example a condensor or a dish.

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[1] Celite® is the tradename of a commercially available product. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

6.7 Drying oven, electrically heated and ventilated, capable of being maintained at a temperature of (130 ± 2) °C.

6.8 Desiccator, containing blue silicagel as desiccant, provided with a perforated plate, preferably of aluminium or stainless steel, of thickness 2 mm to 3 mm.

6.9 Muffle furnace, electrically heated, provided with air circulation and temperature control capable of maintaining the temperature around the crucibles to the nearest 25 °C at temperatures of 475 °C to 525 °C.

The reading of the pyrometer of the muffle furnace cannot always be trusted: deviations may occur. Therefore, the temperature in the muffle furnace should be checked regularly.

Depending on the size and type of the muffle furnace, temperatures in the furnace may differ from one place to another place. When the oven door is closed, adequate air supply should be guaranteed. The volume flow rate of air should not be so great that material is carried away from the crucibles.

6.10 Cold-extraction device, provided with

- a support for the filter crucible (6.3);
- a discharge pipe with a tap to the vacuum and liquid outlet; and
- connecting rings for connecting the filter crucible (6.3).

6.11 Heating device (for manual method), provided with a suitable cooling device capable of maintaining the volume constant during boiling.

6.12 Heating device (for the semi-automatic method) for acid and alkaline digestion, provided with

- a support for the filter crucible (6.3);
- a discharge pipe with a tap to the vacuum and liquid outlet;
- a boiling cylinder of at least 270 ml capacity, with a reflux condenser;
- connecting rings for connecting the heating device to the filter crucible (6.3) and the boiling cylinder; and
- optionally, provision for compressed air.

Before use, preheat the apparatus with boiling water for 5 min.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [6].

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

Using the grinding device (6.1), grind the air-dry laboratory sample so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

For the manual method, proceed in accordance with clause 9.

For the semi-automatic method, proceed in accordance with clause 10.

9 Procedure for manual method

9.1 Test portion

Weigh about 1 g of the prepared test sample (clause 8) to the nearest 0,1 mg (m_1).

If the fat content of the sample exceeds 100 g/kg, or if the sample contains fats which cannot be extracted directly with light petroleum (5.8), transfer the sample to a crucible (6.3) and proceed in accordance with 9.2.

If the fat content of the sample does not exceed 100 g/kg, transfer the sample to a beaker (6.6) and proceed in accordance with 9.3 if the carbonate content, expressed as calcium carbonate, exceeds 50 g/kg. If this is not the case, proceed in accordance with 9.4.

9.2 Preliminary defatting

In the cold-extraction device (6.10), defat the sample three times under vacuum with 30 ml of light petroleum (5.8) each time. Dry the residue by suction after each washing. Transfer the residue to a beaker (6.6).

If the carbonate content, expressed as calcium carbonate, exceeds 50 g/kg, proceed in accordance with 9.3. If not, proceed in accordance with 9.4.

9.3 Removal of carbonate

Pour 100 ml of hydrochloric acid (5.2) over the sample. Stir continuously for 5 min. Carefully pour the mixture into a filter crucible, the bottom of which is covered with a thin layer of filter aid (5.6).

Decant twice with 100 ml of water each time. Take care so that as little material as possible ends up on the filter.

Transfer the contents of the crucible to the original beaker and proceed in accordance with 9.4.

9.4 Acid digestion

Pour 150 ml of sulfuric acid (5.3) over the sample.

Bring to the boil as quickly as possible and continue steady boiling for (30 ± 1) min.

At the beginning of boiling, swirl a few times. If foaming occurs, add a few drops of antifoam agent (5.7). During boiling, maintain a constant volume by using a suitable cooling device (see 6.6 and 6.11).

9.5 First filtration

In the filter crucible (6.3) apply a layer of filter aid (5.6) with a thickness of about one-fifth of the height of the filter crucible. The filter aid may be covered by a sieve plate (6.4) to prevent splashing.

When the boiling time has elapsed, filter the liquid down a stirring rod into the filter crucible. Apply a weak vacuum, so that just in one pass 150 ml is almost completely poured. If the filter blocks, carefully shift aside with a stirring rod the crude fibre covering the filter aid.

Wash the residue five times with about 10 ml of hot water each time. Take care that the filter plate of the crucible remains covered by the filter aid, so that the crude fibre will not reach the filter plate.

Release the vacuum and transfer a volume of acetone (5.5) which is sufficient to just cover the residue. Wait a few minutes and then remove the acetone by applying slight suction. For a few moments suck air through to dry the residue.

If the sample contains fats which cannot be extracted directly with light petroleum (5.8), proceed in accordance with 9.6. If not, proceed in accordance with 9.7.

9.6 Defatting

In the cold-extraction device (6.10), defat the sample three times under vacuum with 30 ml of light petroleum (5.8) each time. Dry the residue by suction after each washing.

9.7 Alkaline digestion

Transfer the residue quantitatively into the same beaker used for the acid digestion.

Add 150 ml of potassium hydroxide solution (5.4) and bring to the boil as quickly as possible. Continue steady boiling for (30 ± 1) min. During boiling, maintain a constant volume by using a suitable cooling device (see 6.6 and 6.11).

9.8 Second filtration

Filter the contents of the beaker through the filter crucible (6.3) containing a layer of filter aid (5.6) with a thickness of about one-fifth of the height of the filter crucible, covered by a sieve plate (6.4) to prevent splashing.

Wash the residue with hot water until the rinsings are neutral.

Wash the residue three times under vacuum with 30 ml of acetone (5.5) each time. Dry the residue by suction after each washing.

9.9 Drying

Place the filter crucible in an incineration dish (6.5) and dry the dish with its contents for at least 2 h in the drying oven (6.7) set at a temperature of 130 °C.

During ashing or cooling, parts of the sintered filter plate of the crucible may come loose. As this may cause an incorrect analysis result, place the filter crucible in an incineration dish.

Leave the filter crucible and the incineration dish to cool in the desiccator (6.8). Immediately after removal from the desiccator, weigh the filter crucible and the incineration dish to the nearest 0,1 mg (m_2).

9.10 Ashing

Place the filter crucible and the incineration dish in the muffle furnace (6.9) and ash its contents at a temperature of (500 ± 25) °C until the difference between two consecutive weighings after cooling does not exceed 2 mg.

After each ashing, leave the filter crucible and the incineration dish to cool partly and, while still warm, place in the desiccator. Leave to cool completely then weigh to the nearest 0,1 mg (m_3).

9.11 Blank determination

Carry out a blank determination as described in 9.4 to 9.10 with about the same quantity of filter aid (5.6) but without the sample.

The loss in mass resulting from ashing (9.10) shall not exceed 2 mg.

Proceed in accordance with clause 11.

10 Procedure for semi-automatic method

10.1 Test portion

Weigh about 1 g of the prepared test sample (clause 8) to the nearest 0,1 mg (m_1). Transfer this quantity to a filter crucible (6.3) provided with about 2 g of filter aid (5.6).

If the fat content of the sample exceeds 100 g/kg, or if the sample contains fats which cannot be extracted directly with light petroleum (5.8), proceed in accordance with 10.2.

If the fat content of the sample does not exceed 100 g/kg, proceed in accordance with 10.3 if the carbonate content, expressed as calcium carbonate, exceeds 50 g/kg. If this is not the case, proceed in accordance with 10.4.

10.2 Preliminary defatting

Connect the filter crucible to the cold-extraction device (6.10) and wash the sample three times under vacuum with 30 ml of light petroleum (5.8) each time. Dry the residue by suction after each washing.

If the carbonate content, expressed as calcium carbonate, exceeds 50 g/kg, proceed in accordance with 10.3. If not, proceed in accordance with 10.4.

10.3 Removal of carbonate

Connect the filter crucible to the heating device (6.12). Wash the sample three times with 30 ml of hydrochloric acid (5.2) each time. After each addition, leave for about 1 min before filtering.

Wash once with about 30 ml of water and proceed in accordance with 10.4.

10.4 Acid digestion

Connect the boiling cylinder to the filter crucible. Transfer 150 ml of boiling sulfuric acid (5.3) to the cylinder with the filter crucible. If foaming occurs, add a few drops of antifoam agent (5.7). Bring to the boil as quickly as possible and boil vigorously for (30 ± 1) min.

10.5 First filtration

Switch off the heating. Open the tap to the discharge pipe and, under vacuum, filter the sulfuric acid through the filter crucible. Wash the residue at least three times with about 30 ml of hot water each time, until neutral. Dry the residue by suction after each washing.

If filtration problems occur, it is recommended to carefully blow air through to remove blockage of the filter.

If the sample contains fats which cannot be extracted directly with light petroleum (5.8), proceed in accordance with 10.6. If not, proceed in accordance with 10.7.

10.6 Defatting

Connect the filter crucible to the cold-extraction device (6.10) and wash the residue three times under vacuum with 30 ml of acetone (5.5) each time. Next, wash the residue three times under vacuum with 30 ml of light petroleum (5.8) each time. Dry the residue by suction after each washing.

10.7 Alkaline digestion

Close the outlet tap. Transfer 150 ml of boiling potassium hydroxide solution (5.4) to the cylinder with the filter crucible. Add a few drops of antifoam agent (5.7). Bring to the boil as quickly as possible and boil vigorously for (30 ± 1) min.

10.8 Second filtration

Switch off the heating. Open the tap to the discharge pipe and under vacuum filter the potassium hydroxide solution through the filter crucible. Wash the residue at least three times with about 30 ml of hot water each time, until neutral. Dry the residue by suction after each washing.

If filtration problems occur, it is recommended to carefully blow air through to remove blockage of the filter.

Connect the filter crucible to the cold-extraction device (6.10) and wash the sample three times under vacuum with 30 ml of acetone (5.5) each time. Dry the residue by suction after each washing.

10.9 Drying

Place the filter crucible in an incineration dish (6.5) and dry the dish with its contents for at least 2 h in the drying oven (6.7) set at a temperature of 130 °C.

During ashing or cooling, parts of the sintered filter plate of the crucible may come loose. As this may cause an incorrect analysis result, place the filter crucible in an incineration dish.

Leave the filter crucible and the incineration dish to cool in the desiccator (6.8). Immediately after removal from the desiccator, weigh the filter crucible and the incineration dish to the nearest 0,1 mg (m_2).

10.10 Ashing

Place the filter crucible and the incineration dish in the muffle furnace (6.9) and ash its contents at a temperature of (500 ± 25) °C until the difference between two consecutive weighings after cooling does not exceed 2 mg.

After each ashing, leave the filter crucible and the incineration dish to cool partly and, while still warm, place in the desiccator. Leave to cool completely then weigh to the nearest 0,1 mg (m_3).

10.11 Blank determination

Carry out a blank determination as described in 10.4 to 10.10 with about the same quantity of filter aid (5.6) but without the sample.

The loss in mass resulting from ashing (10.10) shall not exceed 2 mg.

11 Calculation

Calculate the crude fibre content of the test sample by the equation:

$$w_f = \frac{m_2 - m_3}{m_1}$$

where

w_f is the crude fibre content, in grams per kilogram, of the test sample;

m_1 is the mass, in grams, of the test portion (9.1 or 10.1);

m_2 is the mass, in milligrams, of the incineration dish with the filter crucible with the residue obtained after drying at 130 °C (9.9 or 10.9);

m_3 is the mass, in milligrams, of the incineration dish with the filter crucible with the residue obtained after ashing at (500 ± 25) °C (9.10 or 10.10).

Round the result to the nearest 1 g/kg.

NOTE The result may also be calculated as a mass fraction in percent.

12 Precision

12.1 Interlaboratory tests

Details of interlaboratory tests on the precision of the method are given in annex A. The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

12.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the repeatability limit (r) mentioned in or derived from Table 1.

Table 1 — Repeatability limit (r) and reproducibility limit (R)

Sample	Crude fibre content g/kg	r g/kg	R g/kg
Sunflower seed meal	223,3	8,4	16,1
Palm kernel expellers	190,3	19,4	42,5
Cattle feed (pelleted)	115,8	5,3	13,8
Corn gluten feed	73,3	5,8	9,1
Tapioca	60,2	5,6	8,8
Dog food	30,0	3,2	8,9
Cat food	22,8	2,7	6,4

12.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit (R) mentioned in or derived from Table 1.

13 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- the test result obtained, or the two test results obtained if the repeatability has been checked.

Annex A
(informative)

Results of interlaboratory tests

The precision of the method was established in 1996 and 1997 by four interlaboratory tests carried out in accordance with the split-level experiment of ISO 5725:1986 [2]2) with the exception of application of the Grubbs outlier test instead of the Dixon outlier test. The Grubbs outlier test is described in ISO 5725-2:1994 [4].

In the interlaboratory tests 10 laboratories participated. Samples of sunflower seed meal, palm kernel expellers, cattle feed (pelleted), corn gluten feed, tapioca, dog food and cat food were investigated.

Table A.1 — Statistical results of interlaboratory tests

Parameter	Sample [a]						
	1	2	3	4	5	6	7
Number of laboratories retained after eliminating outliers	10	9	10	10	10	10	10
Mean crude fibre content, g/kg	223,3	190,3	115,8	73,3	60,2	30,0	22,8
Repeatability standard deviation (s_r), g/kg	3,00	6,93	1,89	2,07	2,00	1,14	0,96
Repeatability coefficient of variation, %	1,3	3,6	1,6	2,8	3,3	3,8	4,2
Repeatability limit (r) [$r = 2,8 \times s_r$), g/kg	8,4	19,4	5,3	5,8	5,6	3,2	2,7
Reproducibility standard deviation (s_R), g/kg	5,75	15,18	4,93	3,25	3,14	3,18	2,29
Reproducibility coefficient of variation, %	2,6	8,0	4,3	4,4	5,2	10,6	10,0
Reproducibility limit (R) [$R = 2,8 \times s_R$), g/kg	16,1	42,5	13,8	9,1	8,8	8,9	6,4

[a] 1: sunflower seed meal;
2: palm kernel expellers;
3: cattle feed (pelleted);
4: corn gluten feed;
5: tapioca;
6: dog food;
7: cat food.

Bibliography

[1] ISO 4793:1980, *Laboratory sintered (fritted) filters — Porosity grading, classification and designation.*

[2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests.*

[3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.*

[4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic metod for the determination of repeatability and reproducibility of a standard measurement method.*

[5] ISO 6492, *Animal feeding stuffs — Determination of fat content.*

[6] ISO 6497, *Animal feeding stuffs — Sampling.*

[7] ISO 6541, *Agricultural food products — Determination of crude fibre content — Modified Scharrer method.*

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